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CHARACTERIZATION ON THE CARDIAC FUNCTION OF HYPOTHERMIC DOGS FROM COLD WATER IMMERSION

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OBJECT To clarify the effects of cold water immersion on the canine cardiac function and how to prevent the immersed hypothermic ventricular fibrillation. METHODS 7 normal male dogs, which were randomly divided into two groups, the experimental (3, each with 2.5mg/kg nimodipine) and the control (4), were immersed in cold water at 6 ± 1!. The polygraph system was used to record the cardiac function and the transmission electron microscope was involved to study the ultrastructure changes of myocardium. The activity levels of Na^+-K^+-ATPase and Ca^{2+}-ATPase were measured by means of Elisa. RESULTS As the canine core temperature going down, it was noted that the left ventricular pressure peak value (LVSP), and the isovolumetric contraction phase maximal ascending/descending velocity (± dP/dt_{max}) decreased evidently, with a prolonged Q-T duration, and that hypothermic J waves were found subsequently. The activities of Na^+-K^+-ATPase and Ca^{2+}-ATPase were both inhibited. There came rapid ventricular arrhythmia with the control dogs, while not with the experimental. CONCLUSIONS The hypothermia would result in the weakening of canine cardiac function, moreover, the appearance of J wave might be followed by the occurrence of hypothermic ventricular fibrillation. The results also suggested that nimodipine might have a potential to prevent immersed hypothermic ventricular fibrillation.

Keywords: cardiac function; cold water immersion; hypothermia; nimodipine

Hypothermia is subnormal temperature within the central body. The lowering of the body temperature occurs as the body is robbed of heat by the surroundings. Normal body core temperature is 37.0°C. Shivering and the sensation of cold can begin when the body temperature lowers to approximately 35.8°C [1]. Amnesia can begin to set in at approximately 34.4°C. When the core temperature drops below 32.2°C significant hypothermia begins, with unconsciousness at 30.0°C. Death may occur at approximately 26.1°C.

Immersion hypothermia should be considered part of most dive accidents and the boating accidents. Water conducts body heat away up to 25-26 times faster than air of the same temperature [2]. When a person is immersed in cold water, their skin and nearby tissues cool rapidly, while it may take some minutes before the temperature of the heart and brain starts to drop. Hypothermia may be mild, moderate, or severe. The presentation may range from shivering and piloerection ("goosebumps"), to profound confusion, irreversible coma and death.

On immersion in very cold water, there is sudden hyperventilation, an involuntary gasp, and a varying amount of diving response follows. The diving response consists of a slowing of the heart beat, a decrease or cessation of respiration and a dramatic change in the circulation of the blood with circulation only to the most inner core of the body, the heart, lungs and brain. Then dangerous life-threatening heart rhythms develop which are hard to reverse. Therefore, immersion hypothermia is a severe clinical syndrome [3].

Cold water immersion victims have been fully resuscitated when treated carefully with a variety of rewarming techniques ranging from warm blankets to complete cardiopulmonary bypass tech-

niques in major hospitals. However, once there is a hypothermic ventricular fibrillation, it is hard to have a better treatment. The mortality rate is between 60%-80%. This work focused on the effects of cold water immersion on the canine cardiac function and on the prevention of immersed hypothermic ventricular fibrillation.

1. Materials and methods

1.1. Experimental animals

7 male adult crossbred dogs of 1 to 2 years of age, weighing 20 to 25 kg each, were obtained from the Center of Experimental Animals, INM, China. The dogs were randomly divided into two groups, the experimental (n = 3) that received nimodipine (25 mg/25 ml) via cardiac catheter before immersion and the control (n = 4) that received no treatment. All animals showed normal appearance and activity. Animal surgical procedures and experimental protocol were approved by the Animal Care and Use Committee of INM, China.

1.2. Equipments and reagents

The polygraph system (RM-6000) was from Nihon Kohden; the transmission electron microscope(H-700) was from Japan's Hitachi Company; The UV-VIS spectrophotometer (756MC) was from Shanghai Third Analyses Instrument Factory, and the measuring reagent kits were purchased from Nanking Jiancheng Biological Engineering Research Institute.

1.3. Animal model and Experimental condition

The dogs were anesthetized with 5% pentobarbital sodium (25 mg/kg body weight, *iv*) before the whole body wimmersion in 6 ± 1 ˚C water. The rectal temperature was monitored. The cold water immersion was performed in Low Temperature Environmental Simulation System, INM, China. The water temprature was 6 ± 1 ˚C, while the environmental temprature was 23 ± 1 ˚C.

1.4. Cardiac function measuring

The polygraph system was used to record the cardiac function, including 2-lead electrocardiograms, heart rate, left ventricular pressure peak value (LVSP), isovolumetric contraction phase maximal ascending/descending velocity (± dP/dt_{max}), and hypothermic J waves.

1.5. Myocardial ultrastructure observing

Fresh apical myocardial tissues (1 mm^3) were excised from the anterior wall of the left ventricle at 4˚C after ventricular fibrillation. Tissues were fixed with 3% gluteraldehyde and postfixed with 1% osmium tetroxide. After dehydration with a graded series of ethanol concentrations, the specimens were embedded in epon resin. Ultrathin sections were stained with lead citrate and uranyl acetate. The ultrastructural changes of myocardial tissue were observed under a transmission electron microscope (H.7000, Japan).

1.6. Assay of enzymatic activities

The activity levels of Na^+-K^+-ATPase and Ca^{2+}-ATPase were measured by means of Elisa according to the instruction of the measuring reagent kits.

1.7. Statistical analysis

Data are shown as mean ± SE unless otherwise indicated. Statistical analysis was performed using SPSS11.0 with T test. P-values <= 0.05 were considered significant.

2. Results

2.1. Occurring of ventricular fibrillation

The lead II ECG were recorded before and during cold water immersion. The ECG recorded before immersion was a normal one without J wave (Fig.1A). When the core temprature dropped to 35 ˚C, the physiological indexes showed no significantly abnomity. When the core temprature dropped to 25 ˚C, there came ventricular block, wide QRS, long P-R interval, elevated S-T Segment, and even J wave (Fig. 1B). Then arrhythmia was noted, nor did spontaneous ventricular fibrillation. However, When the core temprature

dropped down to 21 °C, there presented ventricular fibrillation with a reduced intracardiac pressures and a near equalization of diastolic and systolic pressures. All the dogs of the control suffered from ventricular fabrillation at about 21 °C, while those of the experimental did not. The ventricular fibrillation durations were 8, 7, 7 and 5 minutes relatively before the cardiac arrest, while the core tempratures of dogs were 21.3, 20.5, 20.0 and 19.0 °C accordingly. It suggested that short ventricular fibrillation might lead to a cardiac arrest with lower core temprature.

2.2. Myocardial ultrastructure

Under transmission electron microscopy, the myocardial ultrastructure of the dogs with ventricular fibrillation was magnified 5000 and 10000 times, which showed that sarcoplasmic reticulum lightly enlarged in the exterior of muscle bundles. Most mitochondria were found edema-like or collapsed in appearance and some even showed abnormal representation such as vacuolization.

2.3 Change of cordis function indexes.

The RM-6000 polygraph system was used to record the cordis function and the cardiac physiological indexes are listed in Table 1 and 2. Compared with the case itself before and after cold water immersion, LVSP, $\pm dP/dt_{max}$ reduced evidently ($P < 0.05$ or $P < 0.01$), while R-dP/dt_{max} rised evidently ($P < 0.01$). Compared the experimental with the control, there were an obvious discrepancy, and when the core temprature dropped to about 21 °C, there were still an obvious difference with $\pm dP/dt_{max}$.

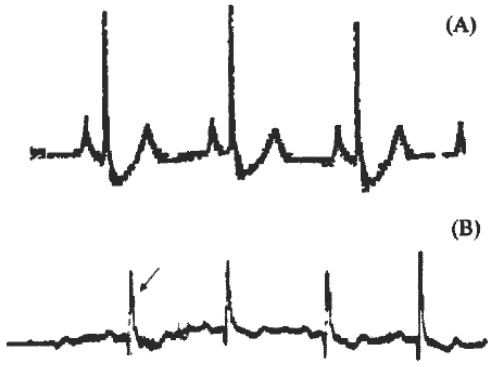

Fig. 1. The lead II ECG before and during cold water immersion. The arrow represents J wave.
(A), ECG before immersion;
(B), ECG at 25 °C core temperature

2.4. Activities of Na^+-K^+-ATPase and Ca^{2+}-ATPase

The activities of Na^+-K^+-ATPase and Ca^{2+}-ATPase were measured by means of Elisa before coldwater immersion and at 21 °C core temprature(Table 3). The data showed that both of the activities of Na^+-K^+-ATPase and Ca^{2+}-ATPase decreased evidently ($P < 0.05$) when immersion hypothermia happened.at 21°C, which implied the two ATPases were inhibited. Compared with the control, the experimental presented that the activities of Na^+-K^+-ATPase descended evidently($P < 0.05$), while the activities of Ca^+- ATPase had no significantly difference($P > 0.05$).

3. Discussion

Hypothermia is commonly found in accidents on land and at sea, which can result from exposure to a cold environment (e.g., accidental drowning) or it can be in-

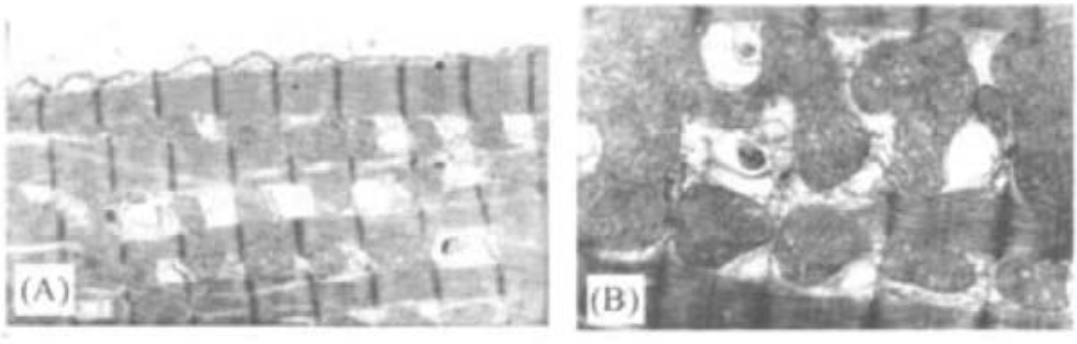

Fig. 2. Myocardial ultrastructure of dogs with ventricular fibrillation
(A), x 5000 times; (B), x 10000 times

Table 1

The cordis function index of the immersed hypothermic dogs (1)($x \pm s$)

Groups	Animals	LVSP(mmHg)				R-dP/dt$_{max}$ (ms)			
		Before immersion	35°C	25°C	21°C	Before immersion	35°C	25°C	21°C
The control	4	172,5 ± 15,0	162,5 ± 16,6	129,25 ± 27*	117 ± 51,86*	0,099 ± 0,037	0,101 ± 0,048	0,164 ± 0,081*	0,195 ± 0,081**
The experimental	3	185,0 ± 25,0	161,7 ± 33,3	126,7 ± 30,55*	93,33 ± 32,15**	0,15 ± 0,04##	0,145 ± 0,022#	0,156 ± 0,026	0,195 ± 0,04*

LVSP: Left ventricular systolic pressure; R-dP/dt$_{max}$: R- maximal velocity of left ventricular pressure

*P < 0.05; **P < 0.01 *vs* before immersion; #P < 0.05; ##P < 0.01 *vs* control

Table 2

The cordis function index of the immersed hypothermic dogs (2)(x ± s)

Groups	Animals	dP/dt$_{max}$ (mmHg/s)				-dP/dt$_{max}$ (mmHg/s)			
		Before immersion	35°C	25°C	21°C	Before immersion	35°C	25°C	21°C
The control	4	64,75 ± 18,9	53,25 ± 19,7	69,5 ± 31,2	20,25 ± 0,5**	39,25 ± 20,1	42,5 ± 28,7	32,25 ± 19,87	13,75 ± 7,5**
The experimental	3	123,33 ± 23,1##	87,33 ± 32,9#	68,33 ± 7,68*	56,67 ± 12,58**#	85,00 ± 21,8##	48,33 ± 16,07	29,0 ± 13,89*	16 ± 6,93**#

± dP/dt$_{max}$: Maximal rise/fall velocity of left ventricular pressure

*P < 0.05; **P < 0.01 *vs* before immersion; #P < 0.05; ##P < 0.01 *vs* control

Table 3

Na^+-K^+/Ca^{2+}-ATPase activities of the hemocytes of the immersed hypothermic dogs (x ± s)

Groups	Animals	Na^+K^+-ATPase (μmol/mg·h)		Ca^{2+}-ATPase (μmol/mg·h)	
		Before immersion	21°C	Before immersion	21°C
Control	4	7,8 ± 2,1	5,4 ± 2,5*	6,9 ± 2,2	5,3 ± 2,2*
Experimental	3	6,7 ± 2,2	3,5 ± 2,1*#	6,9 ± 2,3	4,0 ± 2,1*

*P < 0.05 *vs* before immersion; #P < 0.05 *vs* control

duced and used as a brain protection strategy (e.g., therapeutic hypothermia). The cold water immersion can result in hypotherima, with witch one common ECG presentation is the J wave [4], which is related to the altered cellular activities during hypothermia. In this experiment, there were J waves observed in dogs subjected to cold water immersion. The heart frequently exhibited ventricular arrhythmia after J wave. When the core temprarue descended down to (21 ± 2)!, there came the ventricular fibrillation. Under transmission electron microscopy, the mycardial ultrastructure of the dogs with ventricular fibrillation presented that sarcoplasmic reticulum lightly enlarged in the exterioer of muscle bundles. Most mitochondria were found edema-like or collapsed in appearance and some even showed abnormal representation such as vacuolization. This suggested that cold temprarue might lead injury to the myocardium significantly.

Ventricular fibrillation is a condition in which there is uncoordinated contraction of the cardiac muscle of the ventricles in the heart, making them quiver rather than contract properly. Ventricular fibrillation is a severely abnormal heart rhythm (arrhythmia) that can be life-threatening. The condition results in cardiogenic shock, cessation of effective blood circulation, and sudden cardiac death will result in a matter of minutes. So ventricular fibrillation requires prompt Basic Life Support interventions.

With cold water immersion, the appearance of J wave and the ventricular fibrillation might be relative to Ca^{2+} metabolism, as reported that the reduced sarcoplasmic reticulum Ca^{2+}-ATPase activity is a major determinant of reduced contractility in heart failure [5]. The mechanisms behind this include transient cellular Ca^{2+} overload, or/and reduced availability of Ca^{2+}-ATPase activity or a reduced myofilament responsiveness to Ca^{2+}. Nimodipine is a 1,4-dihydropyridine calcium channel blocker[6]. The contractile processes of smooth muscle cells are dependent upon calcium ions, which enter these cells during depolarization as slow ionic transmembrane currents. Nimodipine inhibits calcium ion transfer into these cells

and thus inhibits contractions of vascular smooth muscle. Although the precise mechanism of action is not known, nimodipine blocks intracellular influx of calcium through voltage-dependent and receptor-operated slow calcium channels across the membranes of myocardial, vascular smooth muscle, and neuronal cells. Nimodipine binds specifically to L-type voltage-gated calcium channels. By inhibiting the influx of calcium in smooth muscle cells, nimodipine prevents calcium-dependent smooth muscle contraction and subsequent vasoconstriction. In view of the interesting combination of pharmacological effects as discussed above, it was decided to test the putative efficacy of nimodipine in hypothermia.

In severe hypothermia, cardiac function variables were demonstrated in intact dogs during surface cooling to 20 to 25°C [7]. In this experiment, the ventricular fabrillation occurred with all the dogs of the control at about 21°C, while it did not with the dogs of the experimental. During cold water immersion, an increase in heart rate was measured, and LVSP, $\pm dP/dt_{max}$ reduced evidently ($P < 0.05$ or $P < 0.01$), while R-dP/dt_{max} and Q-T duration rised evidently ($P < 0.01$), compared with the case itself before and after cold water immersion. Compared the experimental with the control, there were an obvious discrepancy, and when the core temprature dropped to about 21°C, there were still an obvious difference with $\pm dP/dt_{max}$. These observations indicate that cold water immersion hypothermia induced a significant cardiac function variables. By means of Elisa, the activities of Na^+-K^+-ATPase and Ca^{2+}-ATPase were measured. It was found that the activities of both ATPases decreased evidently ($P < 0.05$) at 21°C core temprature, which implied the two ATPases were inhibited. Compared with the control, the experimental presented that the activities of Na^+-K^+-ATPase descended evidently ($P < 0.05$), while the activities of Ca^+-ATPase had decreased but had no significantly difference ($P > 0.05$). The results suggest that nimodipinecan attenuates the activities of Na^+-K^+-ATPase and Ca^{2+}-ATPase. It concluded that nimodipine administration, when administered during the early stage of hypothermia, would prevent immersed hypothermic ventricular fibrillarion (21 °C).

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Резюме

ХАРАКТЕРИСТИКА ФУНКЦІЙ СЕРЦЯ СОБАК ПРИ ГІПОТЕРМІЇ, ВИКЛИКАНІЙ ЗАНУРЕННЯМ В ХОЛОДНУ ВОДУ

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Для уточнення наслідків занурен-

ня в холодну воду на функції серця собак і запобігання викликаної гіпотермією фібриляції шлуночків 7 нормальних псів, які були випадковим чином розділені на дві групи (дослідну і контрольну), були занурені в холодну воду при 6 ± 1 ˚ С. Тварини експериментальної групи попередньо отримали 2,5 мг/кг німодипіну. Для запису функції серця використовували систему поліграф. Для дослідження ультраструктури змін міокарда використовували просвічуючу електронну мікроскопію. Рівні активності Na^+/K^+-АТФази і Ca^{2+}-АТФази були виміряні за допомогою ELISA.При падінні температури тіла собак було відзначено, що пікове значення тиску лівого шлуночка (LVSP) та ізоволюметрічна фаза скорочення максимального зростання/убування швидкості зменшилася. Активності Na^+/K^+-АТФази і Ca^{2+}-АТФази знижувалися. При цьому у контрольних собак спостерігалася, а у досвідчених не спостерігалася швидка шлуночкова аритмія. Показано, що гіпотермія може призвести до ослаблення функції серця собак, крім того, за появою J хвилі може послідувати виникнення викликаної гіпотермією фібриляції шлуночків. Результати дозволяють припустити, що німодипін, можливо, здатний запобігти фібриляції шлуночків, яка викликана гіпотермією.

Ключові слова: функції серця, охолодження зануренням у воду; гіпотермія; німодипин

Резюме

ХАРАКТЕРИСТИКА ФУНКЦИЙ СЕРДЦА СОБАК ПРИ ГИПОТЕРМИИ, ВЫЗВАННОЙ ПОГРУЖЕНИЕМ В ХОЛОДНУЮ ВОДУ

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Для уточнения последствий погружения в холодную воду на функции сердца собак и предотвращения вызванной гипотермией фибрилляции желудочков 7 нормальных кобелей, которые были случайным образом разделены на две группы (опытную и контрольную), были погружены в холодную воду при 6 ± 1 ˚С. Животные экспериментальной группы предварительно получили 2,5 мг/кг нимодипина. Для записи функции сердца использовали систему полиграф. Для исследование ультраструктуры изменений миокарда использовали просвечивающую электронную микроскопию. Уровни активности Na^+/K^+-АТФазы и Ca^{2+}-АТФазы определяли с помощью ELISA. При падении температуры тела собак было отмечено, что пиковое значение давления левого желудочка (LVSP) и изоволюметрическая фаза сокращения максимального возрастания/убывания скорости уменьшилась. Активности Na^+/K^+-АТФазы и Ca^{2+}-АТФазы снижались. При этом у контрольных собак наблюдалась, а у опытных не наблюдалась быстрая желудочковая аритмия. Показано, что гипотермия может привести к ослаблению функции сердца собак, кроме того, за появлением J волны может последовать возникновение вызванной гипотермией фибрилляции желудочков. Результаты позволяют предположить, что нимодипин, возможно, способен предотвратить фибрилляции желудочков, вызванные гипотермией .

Ключевые слова: функции сердца, охлаждение погружением в воду; гипотермия; нимодипин

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